Docket No. 13566.105049

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Bastiaan Nuijen et al.	Conf. No.:	4574
Serial No.:	09/622,433	Group Art Unit:	1655
Filed:	May 10, 2002	Examiner:	Randall O. Winston
For:	PHARMACEUTICAL FORMULATION OF A DIDEMNIN COMPOUND		

DECLARATION OF PRIOR INVENTION UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Bastiaan Nuijen, a citizen of The Netherlands, hereby declare that:

1. I am an inventor of the subject matter disclosed in the above-referenced US Patent Application.

2. I worked with Pharma Mar, S.A., as a collaborator on numerous projects from 1996 to the present, and currently am employed at the Department of Pharmacy & Pharmacology at the Slotervaart Hospital in The Netherlands Cancer Institute, as Head of Pharmaceutical Development & Production. My curriculum vitae listing my professional background, my educational background, and a representative listing of my publications is attached at the end of this Declaration as Exhibit A.

3. I have read the disclosure of US Patent Application Serial number 09/622,433 in its published form as International Patent Application Publication WO 99/42125, which

published on August 26, 1999 ("the '433 application"). I also have reviewed the Office Action issued by the USPTO for the '433 application, dated November 12, 2009 ("the Office Action"), and the publications cited therein, including Crumb et al., U.S. Patent No. 6,030,943 ("Crumb"). I also have reviewed our Response to the Office Action and, in particular, the current Listing of Claims. I understand that claims 1 and 12 of the current Listing of Claims represent the independent claims, which are directed to the subject matter of the invention of the '433 application.

4. It is my belief that the invention described in the current Listing of Claims was actually reduced to practice by at least before May 7, 1997, based for example on my Interim Report of the Pre-Formulation of Aplidine (DDB) ("Interim Report", also "Exhibit B"), which I prepared for a meeting with Pharma Mar. The Interim Report records experiments conducted with the didemnin compound dehydrodidemnin B, which is referred to therein as "Aplidine (DDB)" or simply as "Aplidine".

5. As indicated in the Interim Report, my lab conducted experiments to test the solubility and/or stability of Aplidine in various solutions and at different dilutions in saline (Exhibit B, Tables 1 and 2, pages 3-4). Further, as freeze-dried (lyophilized) formulations are generally more stable, my lab also investigated various solvents in search of a suitable solvent from which Aplidine could be freeze dried. Various candidate freeze-drying solvents were tested (Exhibit B, Tables 3 and 4, page 6-7). A solution composed of Aplidine (DDB), mannitol, and t-butanol in water was found suitable for freeze drying (Exhibit B, Table 5, page 8). This work evidences the actual reduction to practice of "the lyophilized didemnin preparation [which] comprises a didemnin compound and a water-soluble material", according to claim 1; as well as "a didemnin compound" and "a water soluble material" according to claim 12. Further, this

work corresponds to a similar experiment described in the Examples of the '433 application (see page 4 of WO 99/42125).

6. As indicated in the Interim Report, the mannitol and Aplidine freeze-dried product was subsequently found to dissolve in a mixed solvent (co-solvent) system of propylene glycol, ethanol, and water for injection (Exhibit B, Table 6, page 9). This work evidences the actual reduction to practice of "the reconstitution solution of mixed solvents [which] comprises water for injection, an alkanol, and a nonionic surfactant", according to claim 1; and "a nonionic surfactant;" "an alkanol;" and "water for injection" of claim 12, as well as "wherein the water for injection is present in an amount sufficient to allow solubilization of the water soluble material, and the alkanol is present in an amount sufficient to allow solubilization of the didemnin compound", according to both claims 1 and 12. "Cremophor EL" is listed as a component in an "alternative formulation" (Exhibit B, Conclusion, page 11), in place of the nonionic surfactant propylene glycol. Next, my lab examined the stability of the reconstituted preparations at various dilutions with saline (Exhibit B, Table 7, page 10). This work corresponds to a similar experiment described in the Examples of the '433 application (see page 5 of WO 99/42125).

7. Finally, the Interim Report concludes that a "promising formulation of Aplidine (DDB) has been found" (Exhibit B, Conclusion, page 11), ostensibly in terms of the stated goal of formulating a stable "freeze-dried product which can be reconstituted shortly before administration to the patient" (Exhibit B, page 6). These statements evidence the actual reduction to practice of "reconstitution of the lyophilized didemnin preparation with the reconstitution solution of mixed solvents [that] provides a parenterally suitable preparation", in accordance with claim 1.

8. Based on the above, the subject matter of the invention according to independent claims 1 and 12 of the current Listing of Claims for the '433 application was actually reduced to practice by at least before May 7, 1997.

9. Additional laboratory notebook entries further corroborate the actual reduction to practice reported in my Interim Report (Exhibit B). Consider Exhibits C and D, corresponding to two experiments performed in my lab before May 7, 1997, as well as Exhibit E, corresponding to an experiment performed in my lab on that date. The Exhibits include copies of the original laboratory notebook pages, as well as their corresponding English language translations, absent specific dates.

10. Exhibit C, dated less than about one month before my Interim Report, records the results of experiments my lab conducted to test the solubility and stability of Aplidine in various mixed solvent (co-solvent) systems. The entry concludes that "Aplidine has a high solubility (s > 10 mg/mL) in EtOH (ethanol), DMA (dimethylacetamide), PET (peg/ethanol/tween 80), Cremophor EL/EtOH (1:1 v/v) and DMSO (dimethylsulfoxide) but seems only stable on dilution in Cremophor EL/Ethanol during 2 days." Thus, from at least almost a month before my Interim Report, I had the concept of reconstituting Aplidine formulations using mixed solvent systems to achieve stable preparations and, specifically, of using mixed solvent systems involving a nonionic surfactant (Cremophor EL) and an alkanol (ethanol) to do so.

11. Exhibit D, dated a little more than about one month after my Interim Report, records follow up experiments my lab conducted to test the solubility of Aplidine in additional mixed solvent systems composed of a nonionic surfactant, an alkanol, and water. Specifically, my lab tested the solubility Aplidine in "PEN", which is a mixed solvent system of propylene

glycol, ethanol, and water, as well as the solubility in Cremophor EL/ethanol, which is a similar mixed solvent system where Cremophor EL replaces propylene glycol as the nonionic surfactant. The experiments confirmed the suitability of the both mixed solvent systems (see "Conclusion").

12. Exhibit E, dated May 7, 1997, reports additional follow up experiments my lab conducted to test the suitability of mixed solvent systems for reconstituting lyophilized didemnin preparations. Specifically, the experiments investigated the suitability of various mixed solvent systems composed of Cremophor EL, ethanol, and water as the reconstitution solution for various didemnin preparations, including freeze-dried Aplidine (a lyophilized didemnin) and freeze-dried mannitol (a water-soluble material). We determined that lyophilized didemnin preparations can be reconstituted with various Cremophor EL/ethanol/water mixtures, after which the reconstituted preparation can be further diluted without precipitation. These results were further confirmed using HPLC at a later date. This work also corresponds to a similar experiment described in the Examples of the '433 application (see page 4 of WO 99/42125).

13. In sum, the subject matter of the invention according to the current Listing of Claims for the '433 application was actually reduced to practice by at least before May 7, 1997. The idea of reconstituting didemnin preparations using mixed solvent systems including a nonionic surfactant (Cremophor EL) and an alkanol (ethanol) was conceived by at least about one month prior to the actual reduction to practice reported in the Interim Report, and the suitability of various mixed solvent systems were tested and confirmed about one month following the actual reduction to practice reported in the Interim Report, and further on May 7, 1997.

14. All work and experiments referred to herein occurred in The Netherlands, a WTO member country at the time the work and experiments were conducted.

15. All statements made herein are of my own knowledge and true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements or the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any patents arising from United States Patent Application Serial Number 09/622,433.

By: B. Meijer Date: November 8, 2010

Attached: Exhibits A, B, C, D, and E.

EXHIBIT A

CURRICULUM VITAE

Personal

Full name	Bastiaan Nuijen
Date of birth	February 9, 1970
Place of birth	Deventer
Nationality	Dutch
Address	Kapteynstraat 42 2313 RP Leiden, The Netherlands
Telephone	+31 71 514 5528 +31 6 42 42 3973
E-mail	numey@xs4all.nl
Institute	Slotervaart Hospital/Antoni van Leeuwenhoek Hospital (The Netherlands Cancer Institute)
Department	Department of Pharmacy and Pharmacology
Address	Louwesweg 6 1066EC Amsterdam, The Netherlands
Telephone	+31 20 512 4482 (direct), 4481 (secretary)
Fax	+31 20 512 4753
E-mail	Bastiaan.Nuijen@slz.nl

Training

1982-1988	VWO at Gemeentelijke Scholengemeenschap (GSG) (Emmen, The Netherlands)
1994	Masters degree in Pharmacy, Groningen University (Groningen, The Netherlands)
1996	Certified Pharmacist, Groningen University (Groningen, The Netherlands)
1996	Course on Good Manufacturing Practices, Pharmatech Consultancy BV (December 10-12, Bilthoven, The Netherlands)
2000	Ph.D. degree Faculty of Pharmaceutical Sciences, Utrecht University thesis entitled: "Pharmaceutical development of marine-derived anticancer agents", supervisor Prof.Dr J.H.Beijnen; joint-supervisors Prof.Dr A.Bult and Dr H.Talsma (October 26, Utrecht, The Netherlands)
2003	Radiation hygiene-professional level 4B, Inter-faculty Reactor Institute, Delft Technical University (Utrecht, The Netherlands)
2000-2004	Trainee Hospital Pharmacist, Slotervaart Hospital/Antoni van Leeuwenhoek Hospital (supervisor Prof.Dr J.H.Beijnen; Amsterdam, The Netherlands)
2004	Registered Hospital Pharmacist
2004	• Course hospital pharmacists in the Medical Ethical Committee. IMPD steering committee (May 13-14, The Hague, The Netherlands) • 2004 American Association of Pharmaceutical Scientists (AAPS) Annual Meeting and Exposition (November 7-11, Baltimore, USA)
2005	• Workshop risk management: the future of GMP? Pharmaceutical Consultance Services (June 1, Utrecht, The Netherlands) • Training course on quality management in pharma and biotech - Quality and safety of biopharmaceuticals: from genetics to downstream

processing - Biotechnology Studies Delft Leiden (BODL, October 26-28, Delft, The Netherlands)
- 2005 AAPS Annual Meeting and Exposition (November 6-11, Nashville, USA)

2007
- Pharmaceutical Sciences World Congress (PSWC, April 22-25, Amsterdam, The Netherlands)
- European Society of Gene and Cell Therapy (ESGCT) XVth Annual Congress (October 27-30, Rotterdam, The Netherlands)
- Training course on quality management in pharma and biotech – Essentials of integrated GxP in the pharmaceutical industry and hospital pharmacy - BODL (November 5-8, Oegstgeest, The Netherlands)

2008
- 35th Annual Meeting&Exposition of the Controlled Release Society (CRS, July 12-16, New York, USA)
- Training course on quality management in pharma and biotech – Good Quality Management - The role of the Qualified Person - BODL (October 6-8. Noordwijk, The Netherlands)

2009
- 36th Annual Meeting&Exposition of the CRS (July 18-22, Copenhagen, Denmark)
- Training course on Lyophilization Technology – Theory & Practice of Freeze Drying, Center for Professional Advancement (CfPA, June 15-17, Amsterdam, The Netherlands)

2010
- 37th Annual Meeting&Exposition of the CRS (July 17-23, Portland, USA)

Appointments/Positions

1996-2000 Formulation Research Pharmacist, Department of Pharmacy and Pharmacology, Slotervaart Hospital/Antoni van Leeuwenhoek Hospital with main tasks:
- Ph.D. research
- implementation of Good Manufacturing Practices (GMP) and acquiring manufacturer's licence (Fabrikantenvergunning) for the production facility of experimental anticancer agents for clinical use (granted 1999 by Ministry of Health, Welfare and Sport, registration number 101018A)

2000-2004 Trainee Hospital Pharmacist at the Slotervaart Hospital/Antoni van Leeuwenhoek Hospital, Head of Pharmaceutical development and Production

2004-present Hospital Pharmacist at the Slotervaart Hospital/Antoni van Leeuwenhoek Hospital, Head of Pharmaceutical Development and Production

External memberships and committees

2000-present member Dutch Association of Hospital Pharmacists (NVZA)

2002-present member of Medical Ethical Committee Slotervaart Hospital/Jan van Breemen Institute/BovenIJ Hospital

2004-present member American Association of Pharmaceutical Scientists (AAPS)

2008-present member Controlled Release Society (CRS)

Supervision of Ph.D. Research

2002 Joint-supervisor (copromotor) of Bouma, M: Pharmaceutical development of the novel metal-based anticancer agents NAMI-A and AP5280. Utrecht University, September 11, 2002.

2004 Joint-supervisor (copromotor) of den Brok, M.W.J.: Pharmaceutical development of selected novel anticancer agents: from drug substance to final product. Utrecht University, November 3, 2004.

2004 member of thesis committee of Klous, M.G.: Development of diacetylmorphine preparations for prescription to opioid dependent patients. Utrecht University, November 24, 2004.

2006 Joint-supervisor (copromotor) of van der Schoot, S.C.: Pharmaceutical development of investigational anticancer agents: focus on EO-9, AP5346 and GMP implications. Utrecht University, August 30, 2006.

2008 member of thesis committee of Jorritsma-Smit, A.: Immunotherapy of melanoma: towards clinical application. Leiden University, September 4, 2008.

2009 Joint-supervisor (copromotor) of Quaak, S.G.L.: Pharmaceutical development of the plasmid DNA vaccine pDERMATT. Utrecht University, August 28, 2009.

2009 Joint-supervisor (copromotor) of van den Berg, J.H.: Formulation and delivery aspects of dermal DNA vaccines. Utrecht University, November 4, 2009.

Publications

1997

1. J.M.Meerum Terwogt, B.Nuijen, W.W.ten Bokkel Huinink, J.H.Beijnen: Alternative formulations of paclitaxel. *Cancer Treatment Reviews 23: 87-95, 1997*

1999

2. R.W.Sparidans, J.J.Kettenes-van den Bosch, O.van Tellingen, B.Nuyen, R.E.C.Henrar, J.M.Jimeno, G.Faircloth, P.Floriano, K.L.Rinehart, J.H.Beijnen: Bioanalysis of aplidine, a new marine antitumoral depsipeptide, in plasma by high-performance liquid chromatography after derivatization with *trans*-4'-hydrazino-2-stilbazole. *Journal of Chromatography B 729; 43-53, 1999*
3. B.Nuijen, M.Bouma, R.E.C.Henrar, C.Manada, A.Bult, J.H.Beijnen: Compatibility and stability of aplidine, a novel marine-derived depsipeptide antitumor agent, in infusion devices, and its hemolytic potential upon i.v. administration. *Anti-Cancer Drugs 10; 879-887, 1999*

2000

4. B.Nuijen, M.Bouma, R.E.C.Henrar, U.Brauns, P.Bette, A.Bult, J.H.Beijnen: *In vitro* biocompatibility studies with the experimental anticancer agent BIBX1382BS. *International Journal of Pharmaceutics 194; 261-267, 2000*
5. B.Nuijen, M.Bouma, R.E.C.Henrar, P.Floriano, J.M.Jimeno, H.Talsma, J.J.Kettenes van den Bosch, A.J.R.Heck, A.Bult, J.H.Beijnen: Pharmaceutical development of a parenteral lyophilized formulation of the novel antitumor agent

aplidine. *PDA Journal of Pharmaceutical Science and Technology 54 (3); 193-208, 2000*

6. B.Nuijen, M.Bouma, C.Manada, J.M.Jimeno, J.H.M.Schellens, A.Bult, J.H.Beijnen: Pharmaceutical development of anticancer agents derived from marine sources. *Anti-Cancer Drugs 11; 793-811, 2000*

2001

7. B.Nuijen, M.Bouma, J.H.M.Schellens, J.H.Beijnen: Progress in the development of alternative pharmaceutical formulations of taxanes. *Investigational New Drugs 19 (2); 143-153, 2001*
8. B.Nuijen, M.Bouma, C.Manada, J.M.Jimeno, L.Lopez-Lazaro, A.Bult, J.H.Beijnen: Compatibility and stability of the investigational polypeptide marine anticancer agent kahalalide F in infusion devices. *Investigational New Drugs 19 (4); 273-281, 2001*
9. B.Nuijen, M.Bouma, C.Manada, J.M.Jimeno, A.Bult, J.H.Beijnen: In vitro hemolysis and buffer capacity studies with the novel marine anticancer agent kahalalide F and its reconstitution vehicle Cremophor EL/ethanol. *PDA Journal of Pharmaceutical Science and Technology 55 (4); 223-229, 2001*
10. B.Nuijen, M.Bouma, H.Talsma, C.Manada, J.M.Jimeno, L.Lopez-Lazaro, A.Bult, J.H.Beijnen: Development of a lyophilized, parenteral pharmaceutical formulation of the investigational polypeptide marine anticancer agent kahalalide F. *Drug Development and Industrial Pharmacy, 27 (8); 767-780, 2001*
11. B.Nuijen, I.M.Rodrigues-Campos, C.P.Noain, P.Floriano, C.Manada, M.Bouma, J.J.Kettenes-van den Bosch, A.Bult, J.H.Beijnen: HPLC-UV method development and impurity profiling of the marine anticancer agent aplidine in raw drug substance and pharmaceutical dosage form. *Journal of Liquid Chromatography and Related Technologies, 24 (20); 3119-3139, 2001*
12. B.Nuijen, M.Bouma, P.Floriano, C.Manada, H.Rosing, E.Stokvis, J.J.Kettenes-van den Bosch, A.Bult, J.H.Beijnen: Development of a high-performance liquid chromatography method with UV detection for the pharmaceutical quality control of the novel marine anticancer agent kahalalide F. *Journal of Liquid Chromatography and Related Technologies, 24 (20), 3141-3155, 2001*

2002

13. M.Bouma, B.Nuijen, D.R.Stewart, J.R.Rice, B.A.J.Jansen, J.Reedijk, A.Bult, J.H.Beijnen: Stability and compatibility of the investigational polymer-conjugated platinum anticancer agent AP 5280 in infusion solution and its hemolytic potential. *Anti-Cancer Drugs, 13 (9); 915-924, 2002*
14. M.Bouma, B.Nuijen, M.T.Jansen, G.Sava, A.Flaibani, A.Bult, J.H.Beijnen: Photostability profiles of the experimental antimetastatic ruthenium complex NAMI-A. *Journal of Pharmaceutical and Biomedical Analysis, 30 (4); 1287-1296, 2002*
15. M.Bouma, B.Nuijen, G.Sava, A.Perbellini, A.Flaibani, M.J.van Steenbergen, H.Talsma, J.J.Kettenes-van den Bosch, A.Bult, J.H.Beijnen: Pharmaceutical development of a parenteral lyophilized formulation of the antimetastatic ruthenium complex NAMI-A. *International Journal of Pharmaceutics, 248; 239-246, 2002*

16. M.Bouma, B.Nuijen, M.T.Jansen, G.Sava, A.Flaibani, A.Bult, J.H.Beijnen: A kinetic study of the chemical stability of the antimetastatic ruthenium complex NAMI-A. *International Journal of Pharmaceutics, 248; 247-259, 2002*

2003

17. M.Bouma, B.Nuijen, D.R.Stewart, K.F.Shannon, J.V.St.John, J.R.Rice, R.Harms, B.A.J.Jansen, S.van Zutphen, J.Reedijk, A.Bult, J.H.Beijnen: Pharmaceutical quality control of the investigational polymer-conjugated platinum anticancer agent AP5280. *PDA Journal of Pharmaceutical Science and Technology,57; 198-207, 2003*
18. M.Bouma, B.Nuijen, M.T.Jansen, G.Sava, F.Picotti, A.Flaibani, A.Bult, J.H.Beijnen: Develoment of a high-performance liquid chromatography method for pharmaceutical quality control of the antimetastatic ruthenium complex NAMI-A. *Journal of Pharmaceutical and Biomedical Analysis, 31(2); 215-228, 2003*
19. M.W.J.den Brok, B.Nuijen, E.Miranda, P.Floriano, S.Munt, I.Manzanares, J.H.Beijnen: Development and validation of a liquid chromatography-ultraviolet assay using derivatisation for the novel marine anticancer agent (2S,3R)-2-amino-3-octadecanol hydrochloride and its pharmaceutical dosage form. *Journal of Chromatography A, 1020 (2); 251-258, 2003*
20. M.Bouma, B.Nuijen, R.Harms, J.R.Rice, D.P.Nowotnik, D.R.Stewart, B.A.J. Jansen, S.van Zutphen, J.Reedijk, M.J.van Steenbergen, H.Talsma, A.Bult, J.H. Beijnen: Pharmaceutical development of a parenteral lyophilized formulation of the investigational polymer-conjugated platinum anticancer agent AP 5280. *Drug Development and Industrial Pharmacy, 29 (9); 981-995, 2003*

2004

21. M.Bouma, B.Nuijen, E.E.Challa, G.Sava, A.Flaibani, A.Bult, J.H.Beijnen: Stability and compatibility of the investigational antimetastatic ruthenium complex NAMI-A in infuson systems and its hemolytic potential and buffercapacity. *Journal of Oncology Pharmacy Practice, 10; 7-15, 2004*
22. M.W.J.den Brok, S.van der Schoot, B.Nuijen, M.J.X.Hillebrand, J.H.Beijnen: 2-Hydroxypropyl-ß-cyclodextrin extracts 2-phenylphenol from silicone tubing. *International Journal of Pharmaceutics, 278 (2); 303-309, 2004*
23. M.G.Klous, B.Nuijen, W.van der Brink, J.M.van Ree, J.H.Beijnen: Development and manufacture of diacetylmorphine/caffeine sachets for inhalation by "chasing the dragon" by heroin addicts. *Drug Development and Industrial Pharmacy, 30 (7); 775-784, 2004*
24. M.G.Klous, B.Nuijen, W.van der Brink, J.M.van Ree, J.H.Beijnen: Process characterisation, optimisation and validation of production of diacetylmorphine/caffeine sachets: A design of experiments approach. *International Journal of Pharmaceutics, 285; 65-75, 2004*
25. M.G.Klous, B.Nuijen, W.van der Brink, J.M.van Ree, J.H.Beijnen: Pharmaceutical development of an intravenous dosage form of diacetylmorphine hydrochloride. *PDA Journal of Pharmaceutical Science and Technology, 58 (6); 287-295, 2004*

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26. M.W.J.den Brok, B.Nuijen, C.Lutz, H.G.Opitz, J.H.Beijnen: Pharmaceutical development of a lyophilised dosage form for the investigational anticancer agent Imexon using dimethyl sulfoxide as solubilising and stabilising agent. *Journal of Pharmaceutical Sciences, 94(5); 1101-1114, 2005*
27. M.W.J.den Brok, B.Nuijen, R.Harms, J.N.Buluran, M.D.Harvey, C.K.Grieshaber, J.H.Beijnen: Compatibility and stability of the novel anticancer agent C1311 in infusion devices and its in vitro biocompatibility. *Journal of Oncology Pharmacy Practice, 11 (1); 13-19, 2005*
28. M.W.J.den Brok, B.Nuijen, M.J.X.Hillebrand, C.Lutz, H.G.Opitz, J.H.Beijnen: LC-UV method development and validation for the investigational anticancer agent Imexon and identification of its degradation products. *Journal of Pharmaceutical and Biomedical Analysis, 38(4); 686-694, 2005*
29. M.W.J.den Brok, B.Nuijen, E.E.Challa, C.Lutz, H.G.Opitz, J.H.Beijnen: Compatibility and stability of the novel anticancer agent Imexon in infusion devices and its in vitro biocompatibility. *Anti-Cancer Drugs, 16 (7);727-732, 2005*
30. M.W.J.den Brok, B.Nuijen, M.J.X.Hillebrand, C.K.Grieshaber, M.D.Harvey, J.H.Beijnen: Development and validation of an LC-UV method for the quantification and purity determination of the novel anticancer agent C1311 and its pharmaceutical dosage form. *Journal of Pharmaceutical and Biomedical Analysis, 39 (1-2); 46-53, 2005*
31. M.W.J.den Brok, B.Nuijen, E.Millán, C.Manada, J.H.Beijnen: Pharmaceutical development of a parenteral lyophilized formulation of the investigational anticancer agent ES-285.HCl. *PDA Journal of Pharmaceutical Science and Technology, 59 (4); 246-257, 2005*
32. M.G.Klous, G.M.Bronner, B.Nuijen, J.M.van Ree, J.H.Beijnen: Pharmaceutical heroin for inhalation: thermal analysis and recovery experiments after volatilisation. *Journal of Pharmaceutical and Biomedical Analysis, 39; 944-950, 2005*
33. M.W.J.den Brok, B.Nuijen, J.J.Kettenes-van den Bosch, M.J. van Steenbergen, J.N.Buluran, M.D.Harvey, C.K.Grieshaber, J.H.Beijnen: Pharmaceutical development of a parenteral lyophilised dosage form for the novel anticancer agent C1311. *PDA Journal of Pharmaceutical Science and Technology, 59 (5): 285-297, 2005*

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34. M.W.J.den Brok, B.Nuijen, J.L.García, E.Miranda, P.Calvo, C.Manada, J.H.Beijnen: Compatibility and stability of the novel anticancer agent ES-285.HCl formulated with hydroxypropyl-ß-cyclodextrin, in infusion devices and its in vitro biocompatibility. *Pharmazie, 61 (1); 21-24, 2006*
35. S.A.Veltkamp, B.Thijssen, J.S.Garrigue, G.Lambert, F.Lallemand, F.Binlich, A.D.Huitema, B.Nuijen, A.Nol, J.H.Beijnen, J.H.Schellens: A novel self-microemulsifying formulation of paclitaxel for oral administration to patients with advanced cancer. *British Journal of Cancer, 2006*
36. S.C.van der Schoot, B.Nuijen, P.Sood, K.B.Thurmond II, J.R.Rice, J.H.Beijnen: Pharmaceutical development, quality control, stability and compatibility of a parenteral lyophilized formulation of the investigational polymer-conjugated platinum antineoplastic agent AP5346. *Pharmazie, 61 (10); 835-844, 2006*

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37. S.C.van der Schoot, L.D.Vainchtein, J.H.Beijnen, A.Gore, D.Mirejovsky, L.Lenaz, B.Nuijen: EO-9 bladder instillations: formulation selection based on stability characteristics and in vitro simulation studies. *International Journal of Pharmaceutics, 329 (1-2); 135-141, 2007*
38. S.C.van der Schoot, B.Nuijen, A.D.R.Huitema, J.H.Beijnen: Assessment of performance of manufacturing procedures in a unit for production of investigational anticancer agents, using a mixed effects analysis. *Pharmaceutical Research, 24 (3); 605-612, 2007*
39. A.Bins, H.Mallo, J.Sein, C.van den Boogaard, W.Nooijen, F.Vyth-Dreese, B.Nuijen, G.C.de Gast, J.B.A.G.Haanen: Phase I clinical study with multiple peptide vaccines in combination with tetanus toxoid and GM-CSF in advanced-stage HLA-A*0201-positive melanoma patients. *Journal of Immunotherapy, 30 (2); 234-239, 2007*
40. S.C.van der Schoot, B.Nuijen, F.M.Flesch, A.Gore, D.Mirejovsky, L.Lenaz, J.H.Beijnen:. Development of a bladder instillation of the indoloquinone anticancer agent EO-9 using tert-butyl alcohol as lyophilization vehicle. *AAPS PharmSciTech, 8 (3); E61, 2007*

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41. S.C.van der Schoot, B.Nuijen, F.M.Flesch, A.Gore, D.Mirejovsky, L.Lenaz, J.H.Beijnen: Complexation study of the investigational anticancer agent EO-9 with 2-hydroxypropyl-ß-cyclodextrin. *Drug Development and Industrial Pharmacy, 34 (10); 1130-1139, 2008*
42. S.G.L.Quaak, J.H.van den Berg, M.Toebes, T.N.M.Schumacher, J.B.A.G.Haanen, J.H.Beijnen, B.Nuijen: GMP production of pDERMATT for vaccination against melanoma in a phase I clinical trial. *European Journal of Pharmaceutics and Biopharmaceutics, 70 (2); 429-438, 2008*
43. S.C.van der Schoot, L.D.Vainchtein, B.Nuijen, A.Gore, D.Mirejovsky, L.Lenaz, J.H.Beijnen:. Purity profile of the indoloquinone anticancer agent EO-9 and chemical stability of EO-9 freeze dried with 2-hydroxypropyl-ß-cyclodextrin. *Pharmazie, 63; 796-805, 2008*

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November 8, 2010

B. Nuijen

EXHIBIT B

INTERIM REPORT

OF THE

PRE-FORMULATION STUDY OF APLIDINE (DDB)

meeting PHARMAMAR/NDDO-EORTC/NLADF

Department of Pharmacy and Pharmacology
Slotervaart Hospital, Amsterdam

CHARACTERIZATION OF APLIDINE (DDB) BULK DRUG LOT #APL-296

1. IR-SPECTRUM

MAJOR BANDS AT 3320, 2950, 1720, 1630, 1510, 1440 AND 1160 CM^{-1}

SEE FIGURE 1

2. UV/VIS SPECTRUM

ABSORPTION MAXIMA OF APLIDINE IN ETHANOL ABS. (CONC. 63.3 µG/ML) AT λ = 341.8, 273.9 AND 209.9 NM

SEE FIGURE 2

3. NMR AND MASS SPECTROSCOPY

EXPERIMENTS ARE ONGOING

4. HPLC CHROMATOGRAPHY

HPLC SYSTEM:

COLUMN: BECKMAN ULTRASPHERE ODS 5 µM, 4.6 MM X 25 CM
PUMP: MODEL SPECTRASYSTEM P2000 (THERMO SEPARATION PRODUCTS), FLOW 1.0 ML/MIN
INJECTOR: MODEL U6K (WATERS), INJECTION VOLUME 20 µL
DETECTOR: MODEL 996 PHOTODIODE ARRAY DETECTOR (WATERS), λ 225 NM
DATAPROCESSING: MILLENIUM 2010 CHROMATOGRAPHY MANAGER (WATERS)
MOBILE PHASE: A. ACETONITRILE (0.04% TFA), B. WATER (0.04% TFA)
GRADIENT: FROM 45% A TO 85% A IN 30 MINUTES
STOCK: 0.5 MG/ML APLIDINE (DDB) LOT #APL-296 IN ACETONITRIL

THE TEMPERATURE DEPENDENCE OF THE SEPARATION OF THE TWO CONFORMERS OF APLIDINE (DDB) WAS EXAMINED. A COLUMN TEMPERATURE OF 10°C IS SUFFICIENT TO SEPARATE THE CONFORMER-PEAKS (FIGURE 3 AND 4).

SOLUBILITY STUDIES OF APLIDINE (DDB)

THE SOLUBILITY OF APLIDINE (DDB) IN VARIOUS CO-SOLVENT SYSTEMS WAS EXAMINED VISUALLY:

CO-SOLVENT SYSTEM:	**VOLUME CO-SOLVENT ADDED TO 1 MG OF APLIDINE (DDB) LOT #APL-296:**			**SOLUBILITY (MG/ML):**
	100 µL	**900 µL**	**9 ML**	
1. WATER FOR INJECTION	V:- US: -	V:- US:-	V:- US:-	S < 0.1
2. ETHANOL ABS.	V:+ US: X	X	X	S > 10
3. DIMETHYLACETAMDE (DMA)	V:+ US:X	X	X	S > 10
4. PET (POLYETHYLENE GLYCOL/ETHANOL/TWEEN 80 6/3/1 V/V/V)	V:- US:+	X	X	S > 10
5. 0.5% TWEEN 80 IN 0.9% SALINE	V:- US:-	V:- US:-	V:- US:-	S < 0.1
6. DMA/ARACHIS OLEUM	V:- US:-	V:- US:+	X	1 ≤ S < 10
7. CREMOPHOR EL/ETHANOL (1/1 V/V)	V:- US:+	X	X	S > 10
8. DIMETHYLSULFOXIDE (DMSO)	V:- US:+	X	X	S > 10
9.POLYETHYLENE GLYCOL 400 (PEG 400)	V:- US:-	V:- US:+	X	1 ≤ S < 10

-= NOT DISSOLVED
+= DISSOLVED
X= NOT EXECUTED
V= AFTER VORTEXING FOR 30 SECONDS
US= AFTER PLACING IN AN ULTRASONIC BATH FOR 5 MINUTES

TABLE 1

FROM TABLE 1 IT FOLLOWS THAT APLIDINE (DDB) HAS THE HIGHEST SOLUBILITIES IN ETHANOL ABS., DMA, PET, CREMOPHOR EL/ETHANOL AND DMSO (S > 10 MG/ML).

THE STABILITY OF 10 MG/ML SOLUTIONS OF APLIDINE (DDB) IN THESE CO-SOLVENT SYSTEMS WAS EXAMINED ON DILUTION WITH INFUSION FLUID (0.9% SALINE):

10 MG/ML APLIDINE (DDB) IN CO-SOLVENT SYSTEM:	**DILUTION WITH 0.9% SALINE:**				
	1:1	**1:5**	**1:10**	**1:50**	**1:100**
2. ETHANOL ABS.	IP	IP	X	X	X
3. DIMETHYLACETAMDE (DMA)	IP	X	X	X	X
4. PET (POLYETHYLENE GLYCOL/ETHANOL/TWEEN 80 6/3/1 V/V/V)	IP	X	X	X	X
6. DMA/ARACHIS OLEUM	IP	X	X	X	X
7. CREMOPHOR EL/ETHANOL (1/1 V/V)	NP	NP	NP	NP	NP
8. DIMETHYLSULFOXIDE (DMSO)	IP	X	X	X	X
9.POLYETHYLENE GLYCOL 400 (PEG 400)	IP	X	X	X	X

IP= IMMEDIATE PRECIPITATION
NP= NO PRECIPITATION
X= NOT EXECUTED

TABLE 2

TABLE 2 SHOWS THAT ONLY THE 10 MG/ML SOLUTION OF APLIDINE (DDB) IN CREMOPHOR EL/ETHANOL IS STABLE ON DILUTION WITH INFUSION FLUID (0.9% SALINE).

EXAMPLES OF DRUG SUBSTANCES FORMULATED IN CREMOPHOR EL/ETHANOL ARE CYCLOSPORINE (SANDIMMUNE®) AND PACLITAXEL (TAXOL®)

CREMOPHOR EL IS RELATED TO THE OCCURRENCE OF ANAPHYLACTIC REACTIONS ON PARENTERAL ADMINISTRATION. ALSO, INFLUENCE OF CREMOPHOR EL ON THE KINETICS OF DRUGS (FOR INSTANCE PACLITAXEL) IS DESCRIBED. ON THE OTHER HAND, AN ENOURMOUS AMOUNT OF CLINICAL DATA AND EXPERIENCE IS AVAILABLE WITH THIS FORMULATION VEHICLE FOR BOTH TAXOL® AND SANDIMMUNE®.

FREEZE-DRYING OF APLIDINE (DDB)

A SOLUTION OF A DRUG SUBSTANCE IS GENERALLY LESS STABLE IN TIME THAN A FREEZE-DRIED PRODUCT WHICH CAN BE RECONSTITUTED SHORTLY BEFORE ADMINISTRATION TO THE PATIENT. THEREFORE, ATTEMPTS HAVE BEEN MADE TO FREEZE-DRY APLIDINE (DDB) DRUG SUBSTANCE.

THE STARTING-POINT WAS A CONCENTRATION OF 1 MG OF APLIDINE (DDB) PER DOSAGE UNIT.

BECAUSE APLIDINE (DDB) HAS A VERY LOW SOLUBILITY IN WATER (S < 0.1 MG/ML, SEE TABLE 1), AN ADEQUATE SOLVENT HAD TO BE FOUND FROM WHICH APLIDINE COULD BE FREEZE-DRIED.

THE SOLUBILITIES OF APLIDINE (DDB) IN POLYETHYLENE GLYCOL 4000/WATER (E.G. TRIMETAMOL) AND T-BUTANOL/WATER (E.G. BRYOSTATIN 1, RHIZOXIN) SOLVENT SYSTEMS WERE VISUALLY EXAMINED:

SOLVENT:	**VOLUME SOLVENT ADDED TO 1 MG OF APLIDINE (DDB) LOT #APL-296:**				
	100 µL	**1 ML**	**2 ML**	**3 ML**	**4 ML**
10.50% (W/V) POLYE-THYLENE GLYCOL 4000 IN WATER FOR INJECTION	V: - US: -	V: - US: -	V: - US: -	V: - US: -	V: - US: -

-= NOT DISSOLVED
V= AFTER VORTEXING FOR 30 SECONDS
US= AFTER PLACING IN AN ULTRASONIC BATH FOR 5 MINUTES

TABLE 3

FROM TABLE 3 IT FOLLOWS THAT A VOLUME > 4 ML 50% (W/V) POLYETHYLENE GLYCOL 4000 IN WATER FOR INJECTION IS NECESSARY TO DISSOLVE 1 MG OF APLIDINE (DDB). THEREFORE, 50% POLYETHYLENE GLYCOL IN WATER FOR INJECTION SEEMS NO GOOD CANDIDATE AS FREEZE-DRYING SOLVENT.

SOLVENT:	**VOLUME SOLVENT ADDED TO 1 MG OF APLIDINE (DDB) LOT #APL-296:**			**SOLUBILITY (MG/ML)**
	100 µL	**200 µL**	**250 µL**	
11.T-BUTANOL 100%	V: - US: +	X	X	S > 10
12.50% (V/V) T-BUTANOL IN WATER FOR INJECTION	X	V: + US: X	X	S > 5
13.40% (V/V) T-BUTANOL IN WATER FOR INJECTION	X	X	V: + US: X	S > 4

-= NOT DISSOLVED
+= DISSOLVED
V= AFTER VORTEXING FOR 30 SECONDS
US= AFTER PLACING IN AN ULTRASONIC BATH FOR 5 MINUTES

TABLE 4

THE SOLUBILITIES OF APLIDINE (DDB) IN THE EXAMINED T-BUTANOL/WATER SOLVENT SYSTEMS ARE GOOD. 40% (V/V) T-BUTANOL IN WATER FOR INJECTION WAS CHOOSEN AS FREEZE-DRYING SOLVENT.

THE 1 MG/ML APLIDINE (DDB) SOLUTION TO BE FREEZE-DRIED IS COMPOSED AS INDICATED IN TABLE 5. MANNITOL ACTS AS BULKING AGENT.

SUBSTANCE	AMOUNT
APLIDINE (DDB)	25.00 MG
MANNITOL	625 MG
40% (V/V) T-BUTANOL IN WATER FOR INJECTION	Q.S. 25 ML

TABLE 5

THE 1 MG/ML APLIDINE (DDB) SOLUTION WAS PREPARED AS FOLLOWS:

MANNITOL AND APLIDINE (DDB) WERE SUBSEQUENTLY DISSOLVED IN 40% (V/V) T-BUTANOL IN WATER FOR INJECTION. 40% (V/V) T-BUTANOL IN WATER FOR INJECTION WAS ADDED TO VOLUME, AND THE SOLUTION WAS FILTERED THROUGH A 0.22 μM CELLULOSE ACETATE FILTER. 1 ML ALIQOUTS OF THE SOLUTION WERE FILLED INTO 10 ML GLASS VIALS TYPE 1. THE VIALS WERE SEMI-STOPPERED AND PLACED IN THE FREEZE-DRYER. ALL ACTIONS TOOK PLACE UNDER LAMINAR DOWN-FLOW CONDITIONS IN A SAFETY CABINET (CLASS 100) LOCATED IN A CLEAN ROOM ENVIRONMENT.

THE FOLLOWING FREEZE-DRYING CYCLE APPEARED TO BE ADEQUATE:

FREEZING: IN 1 HOUR FROM AMBIENT TO -43°C, FREEZE-HOLD FOR 1.5 HOURS AT -43°C

PRIMARY DRYING: VACUUM OF 1 MBAR IN 1 MINUTE AT -43°C AND HOLDING THIS PRESSURE AND TEMPERATURE FOR 24 HOURS

SECONDARY DRYING: TEMPERATURE RISE FROM -43°C TO 0°C, VACUUM AT 1 MBAR, FOLLOWED BY A TEMPERATURE RISE FROM 0°C TO 25°C IN 0.5 HOUR, VACUUM 1 MBAR, AND TEMPERATURE HOLD DURING 4.5 HOURS

TOTAL FREEZE-DRYING TIME: 35 HOURS

RECONSTITUTION OF FREEZE-DRIED PRODUCT

BECAUSE OF THE PRESENCE OF MANNITOL, THE FREEZE-DRIED CAKE DOES NOT DISSOLVE IN PET OR CREMOPHOR EL/ETHANOL CO-SOLVENT SYSTEMS.

THE SOLUBILITY OF APLIDINE (DDB) FREEZE-DRIED PRODUCT IN PROPYLEENGLYCOL/ETHANOL/WATER FOR INJECTION CO-SOLVENT SYSTEM (E.G. DIGOXIN (LANOXIN®), DIAZEPAM (VALIUM®), TRIMETHOPRIM-SULFAMETHOXAZOLE (SEPTRA®), RHIZOXIN) WAS EXAMINED:

CO-SOLVENT SYSTEM:	**VOLUME CO-SOLVENT ADDED TO APLIDINE (DDB) 1 MG/VIAL FREEZE-DRIED PRODUCT LOT 022697BN2**				
	1 ML	**2 ML**	**3 ML**	**4 ML**	**5 ML**
14.40% PROPYLENE GLYCOL/10% ETHANOL/WATER FOR INJECTION (V/V/V)	V: -	V: -	V: +	V: +	V: +

-= NOT DISSOLVED
+= DISSOLVED
V= AFTER VORTEXING FOR 30 SECONDS

TABLE 6

APLIDINE (DDB) FREEZE-DRIED PRODUCT DISSOLVES IN 3, 4 AND 5 ML OF 40% PROPYLENE GLYCOL/10% ETHANOL/WATER FOR INJECTION CO-SOLVENT SYSTEM (THEORETIC CONCENTRATIONS 0.33, 0.25 AND 0.20 MG/ML OF APLIDINE, RESPECTIVELY). NEXT STEP WAS TO EXAMINE THE STABILITY OF THESE SOLUTIONS ON DILUTION WITH INFUSION FLUID (0.9% SALINE).

CONCENTRATION OF APLIDINE (DDB) IN 40% PROPYLENE GLYCOL/10% ETHANOL/WATER CO-SOLVENT SYSTEM:	DILUTION WITH 0.9% SALINE:						
	1:1	1:2	1:3	1:4	1:5	1:10	1:100
0.33 MG/ML	IP	X	X	X	X	X	X
0.25 MG/ML	NP	NP	NP	NP	NP	NP	NP
0.20 MG/ML	NP	NP	NP	NP	NP	NP	NP

IP= IMMEDIATE PRECIPITATION
NP= NO PRECIPITATION
X= NOT EXECUTED

TABLE 7

AFTER 24 HOURS AT AMBIENT TEMPERATURE, PRECIPITATION HAD OCCURRED IN THE 0.25 MG/ML SOLUTION. THE 0.20 MG/ML SOLUTION SHOWED NO PRECIPITATION, EVEN AFTER 48 HOURS.

THE CLINICAL FEASIBILITY OF 40% PROPYLENE GLYCOL/10% ETHANOL/WATER VEHICLE IS DEPENDENT ON FACTORS LIKE ADMINISTRATION CONDITION, TOTAL DOSE, TARGET POPULATION AND DURATION OF THERAPY. FOR PROPYLENE GLYCOL, CNS DEPRESSION AND CONVULSIONS AFTER INFUSION OF VERY LARGE AMOUNTS (300 ML) WERE REPORTED. A LARGE AMOUNT OF CLINICAL EXPERIENCE IS AVAILABLE WITH THE 40% PROPYLENE GLYCOL/10% ETHANOL/WATER FORMULATION VEHICLE (SEE THE ABOVE MENTIONED EXAMPLES).

CONCLUSION

THE FOLLOWING PROMISING FORMULATION OF APLIDINE (DDB) HAS BEEN FOUND:

PRODUCT: APLIDINE (DDB) 1 MG/VIAL FREEZE-DRIED PRODUCT

RECONSTITUTION SOLUTION: 5 ML 40% PROPYLENE GLYCOL/10% ETHANOL/WATER FOR INJECTION (FINAL CONCENTRATION 0.2 MG/ML)

DILUTION: THE RECONSTITUTED SOLUTION SEEMS STABLE AT LEAST UP TO 100 X DILUTION WITH 0.9% SALINE FOR 48 HOURS

ALTERNATIVE FORMULATION APPROACHES OF APLIDINE (DDB):

CREMOPHOR EL/ETHANOL
LIPOSOMES

EXHIBIT C

Titel: Oplosbaarheidsonderzoek [illegible] (VPB)

Datum: [illegible]

Versie: 1

Doel: oplosbaarheid [illegible] in verschillende co-solvent systemen

Methode: vlgs SOP 'pre-formulation study of [illegible] [illegible] ', [illegible] plasma [illegible]

Resultaten: Afwegen: [illegible] lot ## AR-196.

Uitgangsstof: balans [illegible]

- pipetset [illegible] 7
- micropette M1

1 WFI lot 96102861
2 EtOH Abs. 96090ZKS
3 DMA [illegible] 31912641
4 PET 0309961BN
5 0.5% Tween 80 [illegible] 030996BN
6 DMA/[illegible] 030996BN
7 [illegible]/EtOH (1%) 190197BN
*8 DMSO [illegible] 100g [illegible] 613527BN

NaCl 0.9% [illegible] lot 960[illegible]

2.0.2 zie 002

* extra [illegible]

3		0.00 mg
4	WFI	0.95 mg
5		0.00 mg
6	EtOH	0.95 mg
7		0.00 mg
8		~~0.91 mg~~
9	DMA	1.03 mg
10		0.00 mg
11	PET	1.02 mg
12		0.00 mg
13	0.5% Tween 80 [illegible]	0.99 mg
14		0.00 mg
15	DMA/[illegible]	1.00 mg
16		0.00 mg
17	[illegible]/EtOH	0.98 mg
18		0.00 mg
19	DMSO	0.96 mg

Conclusie: [illegible] heeft [illegible] oplosbaarheid (>10 [illegible]) in EtOH, DMA, PET, [illegible]/EtOH (1%) en DMSO [illegible] [illegible] [illegible] bij [illegible] [illegible] [illegible] 2 [illegible]

Gecontroleerd door: Datum:

Uitgevoerd door: Datum:

Titel: Cyclosporine oculaire Aprene (CPS)

co-solvent systeem	+ 100 µL	+ 900 µL	+ 9 mL	Oplosbaarheid (mg/mL):
1. WFI	V: - US: -	- -	- -	s < 0.1
2. EtOH abs.	V: + US: X	X	X	s > 10
3. DMA	V: + US: X	X	X	s > 10
4. PET	V: - US: +	X	X	s > 10
5. 0.5% Tween in 0.9% NaCl	V: - US: -	- -	- -	s < 0.1
6. DMA/arachis oleum	V: - US: -	+/- +	X	1 ≤ s < 10
7. Cremophor EL/EtOH	V: +/- US: +	X	X	s > 10
8. DMSO	V: +/- US: +	X	X	s > 10

- = NIET OPGELOST
+ = OPGELOST
X = NVT

V = NA VORTEXEN 15 SEC.
US = NA UITKASSEN 10 MIN.

OPM. Alle oplossingen helder en kleurloos behalve cel

VERDUNNING MET 0.9% NaCl:	1:1	1:5	1:10	1:50	1:100
2. EtOH (9.5 mg/mL)	P, na schudden weer in oplossing, opalescent	P	X	X	X
3. DMA (10.3 mg/mL)	P	X	X	X	X
4. PET (10.2 mg/mL)	P	X	X	X	X
6. DMA/arachis oleum (1.00 mg/mL)	P	X	X	X	X
7. Cremophor EL/EtOH (9.8 mg/mL)	IO	IO	IO	IO	IO
8. DMSO (9.6 mg/mL)	P	X.	X	X	X

P = PRECIPITATIE
IO = IN OPLOSSING
X = NVT

Gec

Uitgevoerd door:

Datum:

EXHIBIT D

Titel: Oplosbaarheid DDB tot HAR-297 in PEW en Cremophor EL/EtOH

WEGNO	16
DATUM	
DOEL	Bepalen oplosbaarheid DDB tot HAR-297 in PEW en Cremophor EL/EtOH + na doorverdunnen oplossingen met 0,9% NaCl
METHODE	- Weeg af in een glazen reageerbuis van 10 mL ca 1 mg DDB tot HAR-297

→ PEW (40/10/50 % v/v) (PEW = propyleenglycol/ethanol/water)

Voeg toe achtereenvolgens
1. 1 mL
2. 1 mL (totaal 2 mL)
3. 0.5 mL (totaal 2.5 mL)
4. 0.5 mL (totaal 3 mL)

Vortex na elke toevoeging gedurende 15 sec en beoordeel visueel tegen een zwarte achtergrond de vloeistof op deeltjes; ultrasoon gedurende 5 min en beoordeel de oplossing opnieuw

* Voor PEW (40/10/50 % v/v) is de verwachte oplosbaarheid 1 mg/3.00 mL (z.i.e. verdunning 10 pag 16)

- Weeg af in een reageerbuis glazen van 10 mL ca 1 mg DDB tot HAR-297

→ Cremophor EL/Ethanol (1:1 %)

Voeg achtereenvolgens toe
1. 50 µl
2. 50 µl (totaal 100 µl)

Vortex na elke toevoeging gedurende 15 sec en beoordeel visueel tegen een zwarte achtergrond de vloeistof op deeltjes; ultrasoon gedurende 5 min en beoordeel de oplossing opnieuw

* Voor Cremophor/EtOH (1:1 %) is de verwachte oplosbaarheid 1 mg/100 µl (z.i.e. verdunning 1 pag 2)

→ Voor zowel PEW als Crem/EtOH-oplossingen:

Vervolgens opgelost bekende concentraties worden doorverdund met 0,9% NaCl om precipitatie in infuus te kunnen beoordelen

Gecontroleerd door: Datum:

Uitgevoerd door: Datum:

Titel: Oplosbaarheid DDB lot AAR-297 in PEW en Cremophor EL/EtOH

→ Aan oplossing wordt een gelijk deel (1:1) aan 0.9% NaCl voor infusie toegevoegd gevolgd door vortexen gedurende 15 sec.
Uit de 1:1 verdunning wordt 1 deel genomen en verdund met een gelijk deel 0.9% NaCl (dilution factor 2). Deze handeling wordt uitgevoerd tot dilution factor 1024.
→ Nodig voor zowel PEW als Crem/EtOH 11 glazen buizen 10 mL
Elke verdunning wordt visueel tegen een zwarte achtergrond beoordeeld op precipitatie

Resultaten: Afwegen aan aplidine (DDB) lot AAR-297.

(CE = Cremophor EL, PEW: polyethyleenglycol/ethanol/water 40/10/50 % v/v)

Aplidine (DDB) lot AAR-297

1		0.0 mg
2		0.00 mg
3	CE	0.00 mg
4		1.07 mg
5	PEW	0.00 mg
6		0.95 mg

* PEW 40/10/50 % v/v lot 010497BN.
* Cremophor EL/EtOH (1:1 %).

100 mL bereid als volgt.
Afwegen: 52.538 g Cremophor EL, Sigma lot 16H0493
39.532 g EtOH abs, Baker [illegible]
→ bij elkaar gevoegd en gemengd m.b.v. vortex
→ lot 010497BN

- pipetset 7
- Multipette 1

Gecontroleerd door: Datum:

Uitgevoerd door: Datum:

Titel: Oplosbaarheid DPB lot # APL-297 in PEW en Cremophor EL/EtOH

	1 ML	2 ML	2.5 ML	3.0 ML
PEW V:	–	–	–	–
(90/10/5 0.2 %) VS:	–	–	–	+ *

* na centrifugeren 15 min bij 3000 rpm geen precipitaat [illegible]

	50 µl	100 µl
Cremophor / V:	–	+/– **
Ethanol VS:	+/– **	+
(1:1 %)		

** moeilijk te beoordelen i.v.m. klein volume

* 0.9 % NaCl was [illegible] lot 97C30 SEI

Verdunnings factor	PEW (90/10/5 0.2 %) (0.9 mg/3.0 ml)	Crem/EtOH (1:1 %) (0.9 mg/100 µl)
1	–	+
2	–	+
4	–	+
8	–	+
16	–	+
32	–	+
64	–	+
128	test gestopt	+
256	*	+
512	*	+
1024	*	+

+ = opgelost

– = niet opgelost / precipitatie

* = niet uitgevoerd

Conclusie: Resultaten bevestigen eerdere waarnemingen (PEW precipitatie na verdunning 20 voud 15; goede oplosbaarheid in Crem/EtOH e.o. werk 1)
⇒ waarneming met Cremophor EL/EtOH [illegible] met [illegible] volume oplossing in zeer hoge concentratie (ca. 10 mg/ml)

Gecontroleerd door: Datum:

Uitgevoerd door: Datum:

EXHIBIT E

Titel: Cremophor EL/Ethanol/Water als reconstitutie oplossing voor DPB 1Mg/vial (1

Vervolg	17
Datum	
Doel	Bekijken geschiktheid Cremophor EL/Ethanol/Water-mengsel als reconstitutie oplossing voor gevriesdroogd Aplidine (DPB)
Methode	
	- Streven - concentratie reconstitutie oplossing: 1Mg DPB/ML
	- zo laag mogelijke concentratie Cremophor EL
	- Achtereenvolgens bekijken (visuele beoordeling):
	Oplosbaarheid van - 25 Mg Mannitol
	- 1Mg DPB
	- Gevriesdroogd blanco DPB
	- Gevriesdroogd DPB
	in 1 ML van verschillende Crem. EL/EtOH/water-mengsels
	- I.g.v. oplossing gevriesdroogd product: doorverdunning in 0.9% NaCl voor infusie en visueel bekijken op precipitatie.
Materiaal	- Cremophor EL/Ethanol 50/50 % v/v:
	Cremophor EL 157.50 g Sigma lot 16H0043
	Ethanol abs. 118.64 g Baker lot 8099
	= 300 ML, DOM 070597
	- Gedestilleerd water: lot 970128E1 (SLZ)
	- Gevriesdroogd blanco DPB: lot 041697 BN2
	- Gevriesdroogd DPB 1Mg/vial lot 041697BN3
	- 0.9% NaCl voor infusie lot 97032461
	- Mannitol lot 68-142 A ; - Aplidine (DPB) lot HAPL-297
	Multipette 1, pipetset Pharma 7
	Cremophor EL/Ethanol/Water-mengsels: verhouding C/E t.o.v. W; toevoegen C/E aan W in volgende verhoudingen
	(C/E/W)
	1 50/50 % v/v 4 20/80 % v/v
	2 40/60 % v/v 5 10/90 % v/v
	3 30/70 % v/v 6 60/40 % v/v

Gecontroleerd door: Datum:

Uitgevoerd door: Datum:

Titel: Cremophor EL/ethanol/water als reconstitutievloeistof voor DDB 1mg/ml (1)

Discussion

- voor afgewogen hoeveelheden mannitol en aplidine zie

NO.	CO-SOLVENT SYSTEM Cremophor EL/ethanol/water (C/E/W) (1 mL) C/E + W in x/y% %	DISSOLUTION OF:			
		MANNITOL 25 MG	APLIDINE (DDB) 1 MG	FD MANNITIOL	FD DDB
1	50/50% v/v (1)	V: - US: + M1	V: - US: + A1	X	V: + (n=1)
2	40/60% v/v (2)	V: ± US: + M2	V: - US: + A2	V: + (n=1)	V: + (n=1)
3	30/70% v/v (3)	V: ± US: + M3	V: - US: + A3	X	V: + (n=2)
4	20/80% v/v (4)	V: + M4	X	X	V: + (n=1)
5	10/90% v/v (5)	V: + M5	V: - US: ± A5	X	V: + (n=2)
6	60/40% v/v (6)	X	V: - US: + A6	V: + (n=1)	X

K

NO.	DILUTION FACTOR										
	1	2	4	8	16	32	64	128	256	512	1024
3	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+

+ = Dissolution/precipitation
- = No dissolution/precipitation
V = Vortexing 25 sec.
US = Ultrasonic bath 5-10 min.
X = Not executed

Gecontroleerd door: Datum:

Uitgevoerd door: Datum:

Titel: Cremophor EL/ethanol/water als reconstitutie oplossing voor DDB 1mg/vial (1)

[illegible] mannitol / [illegible]:

Mannitol lot 68-142A

DDB lot #MC-297

3		0.00 mg	13		0.00 mg
4	M1	26.04 mg	14		0.00 mg
5		0.00 mg	15		0.97 mg
6	M2	24.59 mg	16	A1	1.04 mg
7		0.00 mg	17		0.00 mg
8	M3	25.57 mg	18	A2	1.00 mg
9		0.00 mg	19		0.00 mg
10	M4	25.92 mg	20	A3	0.95 mg
11		0.00 mg	21		0.00 mg
12	M5	25.41 mg	22	A4	0.96 mg
			23		0.00 mg
			24	A5	0.98 mg

Conclusie: DDB 1mg/vial lijkt gereconstitueerd te kunnen worden met C/E/W mengsel (tot C/E:W 10:90 % v/v) en [illegible] niet [illegible] [illegible] te kunnen worden. → bevestigen resultaten middels HPLC

Gecontroleerd door: Datum:

Uitgevoerd door: Datum: